WO 2004/039386 PCT/HR2003/000049

PATENT CLAIMS

- 1. Method for isolation of sylimarin from Silybum Marianum without precooling seeds, comprising the following steps:
 - a) grinding the seeds to a fine powder
 - b) defatting the pulverized seeds with hydrocarbon solvent
 - c) extraction of defatted seeds with medium polarity solvent at the temperature from 18 to 56° C in order to obtain sylimarin extract
 - d) evaporation of the extract from step c)
 - e) removal of water from the evaporation residue from step d)
 - f) purification of dry extract from the previous step from residual oil
 - g) separation, washing and drying of the obtained crystals
- 2. Method for isolation of sylimarin from *Silybum marianum* according to the claim 1, characterized by the fact that grinding is run in a mill with rotating knives and screen of up to 40 mesh.
- 3. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that grinded seeds is removed into a reactor with mechanical mixer whose form is following geometry of reactor's plate.
- 4. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claime 3, characterized by the fact that defatting is done in an extractor at the temperature at which solvent returns.
- 5. Method for isolation of sylimarin from Silybum marianum seeds according to the claim 1, characterized by the fact that defatting is done in percolator at the room temperature.
- 6. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that hexane and petrol ether are used as solvents for defatting.
- 7. Method for isolation of sylimarin from Silybum marianum seeds according to claim 6, characterized by the fact that n-hexane is used as solvent for defatting.
- 8. Method for isolation of sylimarin from Silybum marianum seeds according to the claim 7, characterized by the fact that ratio between the seeds and n-hexane is from 1:2 to 1:5 m/V.
- 9. Method for isolation of sylimarin from Silybum marianum seeds according to the claim 8, characterized by the fact that ratio between the seeds and n-hexane is 1:3 m/V.

WO 2004/039386 PCT/HR2003/000049

10. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that acetone is used as medium polarity solvent for the extraction of defatted seeds.

- 11. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that the extraction of defatted seeds is done in an extractor during 24 hours, at the approx. temperature at which acetone returns.
- 12. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that the extraction of defatted seeds is done in percolator during 72 hours, at the room temperature.
- 13. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 10, characterized by the fact that ratio between the seeds and acetone is from 1:2 to 1:5 m/V.
- 14. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 13, characterized by the fact that ratio between the seeds and acetone is 1:3 m/V.
- 15. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that evaporated acetone residue is dried by azeotropic distillation with toluene.
- 16. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that purification of dry extract from claim the 15 is done by ethers with 4 to 8 C atoms.
- 17. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 16, characterized by the fact that purification of sylimarin is done approx. at the melting temperature of used ether.
- 18. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 16, characterized by the fact that purification of dry extract is done with tetrahydrofuran, diisopropyl ether or diethyl ether.
- 19. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 18, characterized by the fact that purification of dry extract is done with disopropyl ether at the temperature of approx. 67 to 69 C, during 25 to about 30 minutes.
- 20. Method for isolation of sylimarin from Silybum marianum seeds according to the claim 1, characterized by the fact that suspension is cooled to the room temperature, during 1 hour.
- 21. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that crystales of purified sylimarin are filtered and several times washed with disopropyl ether.

WO 2004/039386 PCT/HR2003/000049

22. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that product is dried in drying section.

- 23. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 22, characterized by the fact that product is dried in drying section at increased temperature and decreased pressure.
- 24. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 17, characterized by the fact that product is dried in drying section at 40° C and the pressure of 8 to about 10 mbar.